

Thrombotic events associated with low baseline direct oral anticoagulant levels in atrial fibrillation: the MAS study

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Key Points

- A relationship between low baseline DOAC levels and thrombotic events in 1-year follow-up was found.
- Early measurement allows the identification of patients with low DOAC levels and treatment adjustment to avoid future thrombotic events.

Although effective and safe, treatment with direct oral anticoagulants (DOAC) in atrial fibrillation (AF) is still associated with thrombotic complications. Whether the measurement of DOAC levels may improve treatment efficacy is an open issue. We carried out the observational, prospective, multicenter Measure and See (MAS) study. Blood was collected 15 to 30 days after starting DOAC treatment in patients with AF who were followed-up for 1 year. Plasma samples were centralized for DOAC level measurement. Patients' DOAC levels were converted into drug/dosage standardized values to allow a pooled analysis in a time-dependent, competitive-risk model. The measured values were transformed into standardized values (representing the distance of each value from the overall mean) by subtracting the DOAC-specific mean value from the original values and dividing by the standard deviation. Trough and peak DOAC levels were assessed in 1657 and 1303 patients, respectively. In total, 21 thrombotic complications were recorded during 1606 years of follow-up (incidence of 1.31% of patients per year). Of 21 thrombotic events, 17 occurred in patients whose standardized activity levels were below the mean of each DOAC (0); the incidence was the highest (4.82% of patients per year) in patients whose standardized values were in the lowest class (-1.00 or less). Early measurement of DOAC levels in patients with AF allowed us to identify most of the patients who, having low baseline DOAC levels, subsequently developed thrombotic complications. Further studies are warranted to assess whether thrombotic complications may be reduced by measuring baseline DOAC levels and modifying treatment when indicated. This trial was registered at www.ClinicalTrials.gov as #NCT03803579.

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Raw data and scripts used for analysis are available upon request to the authors at the Center for Open Science, Charlottesville, VA; <https://osf.io>. Original data are available on request from the author, Cristina Legnani (c.legnani@fondazionearianna.org).

The full-text version of this article contains a data supplement.

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Introduction

Over the last years, clinical trials, meta-analyses, and clinical practice confirmed the efficacy and safety of direct oral anticoagulants (DOACs) for stroke prevention in patients with nonvalvular atrial fibrillation (AF).¹⁻⁸ Recent observational studies in patients with AF showed that DOACs, compared with warfarin, had lower rates of stroke, systemic embolism, comparable rates of major bleedings,⁹ and advantages in terms of risk reduction of intracranial bleeding and systemic embolism, even in older and frail populations.^{10,11} However, a nonnegligible incidence of thrombotic and bleeding events has been recorded in clinical trials and in observational studies in patients receiving DOAC.^{5,7,8} Hence, the issue of improving the clinical management of patients treated with DOACs and further reducing the risk of complications during treatment is relevant.

DOACs are administered to patients with AF based on patient characteristics such as age, comorbidity, body weight, and renal function without dose adjustment based on DOAC concentration measurements. This is mainly based on results of pharmacokinetic studies, which indicated a predictable anticoagulant response and an effective prevention of excessive drug concentration.¹²⁻¹⁶ Furthermore, the registration trials were conducted at dose regimens adjusted for some patient characteristics or for concomitant use of associated interacting drugs, and not for measured DOAC levels.

Consequently, the measurement of DOAC levels has been recommended only in particular situations, such as bleeding or thrombotic complications, before urgent need of surgery or invasive procedures, use of antidotes, and also suggested in special patient populations, such as those with frailty, those who are underweight or overweight, or treated for epilepsy.¹⁷⁻¹⁹ However, studies focusing on the measurement of plasma DOAC levels showed high interpatient variability for all the DOACs and for all the doses used to treat patients.²⁰⁻²⁴

Moreover, postmarketing studies showed that the variability of DOAC levels was even higher than those reported in phase 2 and 3 studies.²⁵⁻²⁹ Indeed, observational studies reported that some of the patients treated at fixed doses may have relatively high or low DOAC plasma levels, thus supporting the issue of assessing whether an early or periodical measurement might contribute to improving the quality of treatment and reducing risks of complications.^{30,31}

In addition, very recent studies showed a relationship between low DOAC levels (generally measured after the events), the risk of ischemic stroke and its severity,³² and the risk of stroke recurrences.³³ Finally, a pilot prospective multicenter study evidenced low plasma levels of DOACs in patients with AF, measured at the time of embolic stroke.³⁴ Whether DOAC concentration measurement may be useful to better tailoring the dose and optimizing the risk-benefit of treatment remains an unsolved clinical problem.

The present study aimed to investigate whether low DOAC plasma levels, assessed at steady-state within the first month of treatment, are associated with thrombotic events during a 1-year follow-up.

Material and methods

The Measure and See study (MAS; ClinicalTrials.gov identifier: NCT03803579) is an observational, prospective cohort, multicenter study of patients with AF, who started treatment with 1 of the available DOACs (dabigatran, apixaban, edoxaban, and rivaroxaban) for therapy and prevention of thrombotic complications. The study was promoted and funded by the Arianna Anticoagulazione Foundation (Bologna, Italy) and conducted in anticoagulation clinics affiliated with the Italian Federation of Anticoagulation Centers.

Patient population

Consecutive patients with AF, without rheumatic mitral valve disease or mechanical heart valves, aged >18 years, seen at the anticoagulation clinics from 27 August 2018 to 10 November 2022, who had started anticoagulation with a DOAC within 1 month, were enrolled in the study. Patients suitable for electrical cardioversion, those who had refused blood sampling, those who did not accept follow-up for at least 1 year, and those who had other clinical indications for anticoagulant therapy were excluded from the study.

The choice of DOAC and dose used was left to the discretion of treating physicians. The study protocol included a recommendation for the participant centers to include patients who were treated following the rules defined for each drug by the Italian regulatory medicines agency (Agenzia Italiana del Farmaco) and current clinical practice.

Each patient was given a unique anonymous identifying code to ensure anonymity, which was used to collect clinical information and identify biological samples. The following baseline characteristics were recorded in a specific electronic database: patient identification number, date of birth, sex, type of drug used and dose, weight, body mass index (BMI), kidney function (estimated by creatinine clearance according to the Cockcroft-Gault formula), liver enzyme function (assessed by aspartate aminotransferase and alanine aminotransferase), diabetes, CHA₂DS₂-VASC (congestive heart failure, hypertension, age ≥75 [doubled], diabetes, stroke [doubled], vascular disease, age 65 to 74 and sex category [female]) score, previous stroke/transient ischemic attack (TIA), other comorbidities, and concomitant medications, (with particular attention to antiplatelet drugs). Data were stored in the database located at a section of the Aruba cloud rented by the Arianna Anticoagulazione Foundation, which guaranteed the database's storage, backup, and maintenance.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Independent review board approval was obtained before all study-related activity from the ethics committee of the coordination center (Cremona; approval no. 14725; 02/05/2018) and from the ethics committees of all other centers. Written informed consent was obtained from each patient before enrollment. The study promoter provided measures to safeguard patient privacy and the protection of personal data according to the European Union General Data Protection Regulations 2016/679 and Italian law.

Blood sampling and DOAC measurement

The study required a mandatory plasma collection to measure the DOAC level for each enrolled patient. Venous blood sampling had

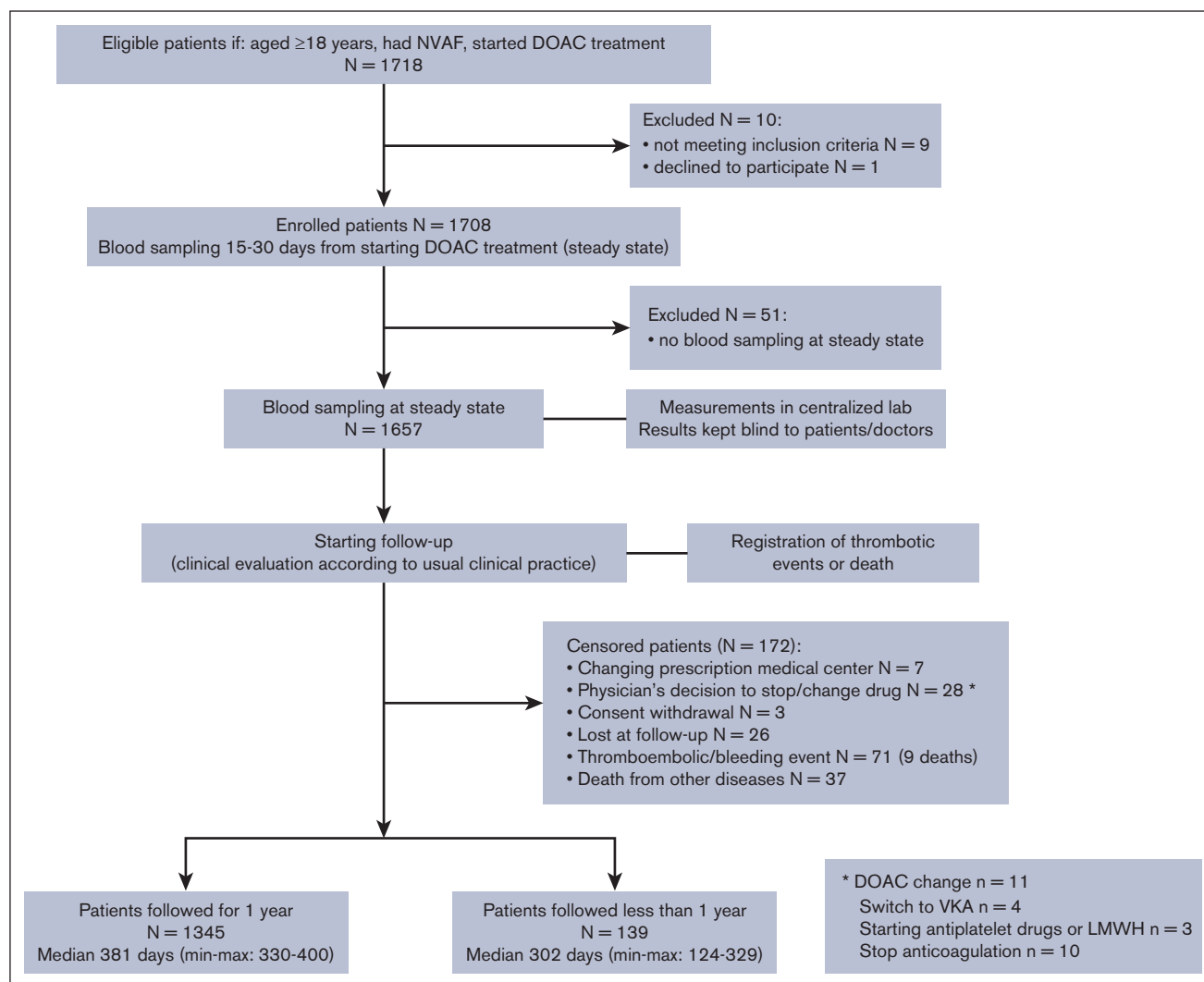


Figure 1. Flowchart of the study. NVAf, nonvalvular atrial fibrillation; VKA, vitamin K antagonist.

to be performed at a steady state (within the first 2-4 weeks of initiation of treatment) and obtained at the time of trough (C-trough), immediately before the subsequent drug intake. It was up to the discretion of participating centers, depending on their availability and organization, to perform additional blood collection on the same day, 2 hours after the last intake (C-peak). Further blood samples were used to perform ancillary laboratory tests (including blood cell count, creatinine clearance, and liver enzymes). Plasma samples for DOAC measurement were collected in vacuum tubes (Vacutainer; Franklin Lakes, NJ) containing 1-in-10 volumes of 3.2% trisodium citrate. Blood was centrifuged within 1 hour of collection at 2000g for 20 minutes (controlled room temperature), and plasma samples were aliquoted in cryovials, identified locally to maintain patient anonymity, in volumes that allow for optimal DOAC testing; sample vials were stored frozen (-80°C)³⁵ at the participating centers and later centralized to the biobank of the Arianna Anticoagulazione Foundation (Bologna). Finally, aliquots were transferred (dry-ice and express courier) to the Haemostasis and Thrombosis Center of Cremona Hospital for DOAC measurement.

DOAC levels, expressed as drug concentration-equivalent (ng/mL), were measured by chromogenic assays using STA-ECA II (Diagnostica Stago, Asnieres-sur-Seine, France) for dabigatran, and STA-Liquid anti-Xa (Diagnostica Stago) for apixaban, edoxaban, and rivaroxaban (hemolyzed samples were discarded).³⁶⁻³⁸ Tests were calibrated using commercial plasmas with certified DOAC concentration supplied by the same manufacturer and performed on STA-R Max instrument (Diagnostica Stago). The results of DOAC levels for each patient were transmitted to the central database repository and were not communicated to patients, participating centers, or attending physicians.

Follow-up and outcomes

Participating centers were instructed to organize blood sampling and clinical follow-up as requested by the study. Follow-up, as defined by Italian Federation of Anticoagulation Centers guidelines, included a clinical evaluation within the first month of treatment and a clinical check-up every 3 to 4 months for 1 year. All thromboembolic and bleeding complications, death, and other events were recorded during the 12-month follow-up.

Table 1. Baseline characteristics of included patients

Patients, n	1657
Participating centers, n	27
Age, median (min-max), y	80 (47-100)
Males, n (%)	896 (54.1)
BMI, median (min-max)	26.2 (14.9-68.1)
Hemoglobin, median (min-max), g/dL	13.3 (8.0-18.8)
Platelets, median (min-max), $\times 10^3/\mu\text{L}$	218 (52-700)
Creatinine clearance, median (min-max), mL/min	58.0 (13-246)
History of cerebrovascular ischemic disease/ peripheral arterial emboli, n (%)	186 (11.2)
History of cardiovascular disease, n (%)	284 (17.1)
History of gastrointestinal bleeding, n (%)	22 (1.3)
History of cancer, n (%)	227 (13.7)
Hypertension, n (%)	1472 (88.8)
Diabetes, n (%)	375 (22.6)
Liver cirrhosis, n (%)	14 (0.8)
Chronic kidney disease, n (%)	197 (11.9)
Hypothyroidism/hyperthyroidism, n (%)	165 (10.0)/62 (3.7)
Smokers, n (%)	190 (11.5)
Alcohol intake, n (%)	58 (3.5)
Mental disorders, n (%)	52 (3.1)
Family/social support, n (%)	1410 (85.1)
Drug daily dose, n (%)	
Apixaban (standard dose)	521 (31.4) [336 (65.5)]
Dabigatran (standard dose)	221 (13.3) [100 (45.3)]
Edoxaban (standard dose)	583 (35.2) [283 (48.6)]
Rivaroxaban (standard dose)	332 (20.0) [238 (71.7)]
Prescribing accuracy of DOACs¹⁹:	
Appropriate, n (%)	1441 (87.0)
Prior VKA treatment, n (%)	512 (30.9)
Use of antiplatelet drugs, n (%)	382 (23.0)
Number of associated drugs, median (min-max)	
Antihypertensives, n (%)	933 (56.3)
Antiarrhythmics, n (%)	695 (41.9)
Gastroprotectors, n (%)	655 (39.5)
Antidyslipidemics, n (%)	585 (35.3)
Thyroid disease drugs, n (%)	210 (12.7)
Anxiolytics, n (%)	175 (10.6)
Psychotropics, n (%)	137 (8.3)
Painkillers, n (%)	67 (4.0)
Steroids, n (%)	47 (2.8)
Antiepileptic drugs, n (%)	28 (1.7)
Nitrates, n (%)	13 (0.8)
Immunosuppressants, n (%)	11 (0.7)
Antivirals, n (%)	7 (0.4)
Polytherapy ≥ 3 , n (%)	1196 (72.2)
CHA ₂ DS ₂ VASc score, median (min-max)	4 (0-8)
CHA ₂ DS ₂ VASc score ≥ 4 , n (%)	1072 (64.7)

min, minimum; max, maximum; VKA, vitamin K antagonist.

The predefined study outcomes were all thromboembolic complications, including objectively documented ischemic cerebral vascular events, systemic emboli, the occurrence of acute venous thromboembolism (VTE), acute myocardial infarction, and thrombotic and cardiovascular deaths. An independent adjudication committee, that was unaware of patient name, the results of DOAC levels in the collected samples, and the enrolling center, assessed all of the adverse events occurring during follow-up. This assessment reports analyses of the data regarding the relationship between DOAC levels and the occurrence of thromboembolic complications during follow-up. The relationship between DOAC levels and bleeding events will be analyzed and reported separately.

Statistical analysis

Sample size. Based on previous studies,^{12,25} we hypothesized that the annualized risk of the primary study outcome may be fourfold higher in the lowest quintile of DOAC distribution than in the highest quintile of DOAC distribution. We assumed an annualized risk of primary study outcome of 1.25% of patients per year (pt/y), and 5% pt/y in patients in the highest and lowest quintile of DOAC plasma concentration, respectively. Under this assumption, a total sample size of 1315 patients would be sufficient to refute a null hypothesis of equivalence with α and β errors of 0.05 and 0.2, respectively.

Analysis plan. Because the absolute C-trough and C-peak DOAC plasma concentrations are drug- and dose-dependent, we standardized each measured absolute value using their drug- and dose-specific mean and standard deviation. Standardized values represent the distance of each value from the drug mean distribution and may be, therefore, pooled to evaluate the effect of drug levels, irrespective of the DOAC type and administration (once or twice a day). Supplemental Figure 1 shows the correlation between unstandardized (absolute) and standardized plasma drug concentrations.

The outcome incidence rates were computed for all patients with at least 1 measured plasma DOAC concentration (C-trough or C-peak). Patients were censored at the end of the study or after the occurrence of a qualifying event. For the primary analysis, we used a Cox regression model allowing for competing risk according to the method by Fine and Gray³⁹ to model the occurrence of the primary study outcomes as a function of standardized drug concentration as a continuous value. The regression model included as possible confounders CHA₂DS₂-VASc score, BMI, creatinine clearance, concomitant antiplatelet use, use of low-dose DOAC, and enrolling center. Deaths occurring for causes other than thromboembolic events were considered as competing events. Akaike information criteria was used to evaluate the goodness of fit of the Fine and Gray model. As a sensitivity analysis, we also explored the risk of the primary study outcomes in patients with values below the first quintile of the absolute drug concentration, after adjustment for the aforementioned possible confounders.

As an exploratory analysis, we evaluated the incidence of the primary thrombotic outcome stratified by standardized values. For this analysis, Kaplan-Meier survival curves were plotted to estimate the cumulative incidence of thrombotic outcomes in patients with

Table 2. Details of all thrombotic outcomes

Sex/ age	Thrombotic outcome	DOAC dose	Weight (kg)	BMI	Creatinine (mg/dL)	Creatinine clearance (mL/min)	C-trough level (ng/mL)	C-peak level (ng/mL)	CHA ₂ DS ₂ VASc score	Previous stroke/TIA	Inappropriate DOAC prescription	Use of antiplatelet drugs	Use of interfering drugs
M/78	Stroke	Apixaban 5 mg BID	90	24.9	1.31	69	94	182	4	Yes	No	No	No
F/78	Stroke	Apixaban 2.5 mg BID	41	18.2	1.31	23	73	84	7	Yes	Yes	No	No
M/71	SVT	Apixaban 5 mg BID	84	25.6	1.20	67	110	150	4	No	No	Yes	No
F/83	Fatal AMI	Apixaban 2.5 mg BID	46	18.7	0.69	44	33	219	4	No	No	No	Yes
M/86	AMI	Apixaban 5 mg BID	86	29.7	1.08	58	106	238	4	No	No	No	No
M/82	AMI	Apixaban 5 mg BID	78	26.4	1.00	63	169	368	4	No	No	Yes	No
M/83	AMI	Apixaban 2.5 mg BID	112	34.6	1.74	50	97	NA	5	No	No	No	No
M/78	Fatal AMI	Apixaban 5 mg BID	66	23.4	1.24	45	32	80	6	No	No	Yes	No
M/62	Stroke	Dabigatran 150 mg BID	85	28.7	0.85	108	68	248	2	No	No	No	No
M/81	DVT	Dabigatran 110 mg BID	73	28.5	1.27	47	90	165	5	Yes	No	No	No
M/67	AMI	Dabigatran 110 mg BID	83	28.1	1.30	65	37	60	3	No	No	Yes	No
M/66	AMI	Dabigatran 150 mg BID	102	32.9	1.37	75	34	NA	4	No	No	Yes	No
M/89	AMI	Dabigatran 110 mg BID	67	21.9	0.91	51	128	NA	3	No	No	No	No
F/83	Stroke	Edoxaban 30 mg	54	23.1	0.75	48	23	101	5	Yes	No	No	No
F/79	Stroke	Edoxaban 30 mg	67	26.2	0.87	55	35	102	5	No	Yes	No	No
M/79	AMI	Edoxaban 30 mg	60	18.5	1.44	35	20	247	5	No	No	Yes	No
M/70	Fatal AMI	Edoxaban 60 mg	64	26.6	1.10	57	40	296	4	No	No	Yes	No
F/72	Fatal AMI	Edoxaban 60 mg	72	28.8	1.00	57	52	NA	3	No	No	No	No
F/79	Fatal stroke	Rivaroxaban 15 mg	55	22.9	0.60	63	17	121	5	No	Yes	No	No
F/89	TIA	Rivaroxaban 15 mg	75	29.3	1.15	39	13	9	5	No	No	Yes	No
M/81	Fatal AMI	Rivaroxaban 20 mg	104	34.0	0.57	150	43	189	6	No	No	Yes	No

AMI, acute myocardial infarction; BID, twice a day (bis in die); DVT, deep vein thrombosis; NA, not available; SVT, superficial vein thrombosis; TIA, transient ischemic attack.

Table 3. Effect of standardized plasma DOAC levels on the primary thrombotic outcome end point, adjusted for potential confounders

Characteristic	First model (C-trough), n = 1657		Second model (C-peak), n = 1298	
	HR	95% CI	HR	95% CI
Standardized C-trough DOAC	0.56	0.37-0.86	-	-
Standardized C-peak DOAC	-	-	0.19	0.06-0.66
CHA ₂ DS ₂ VASc score	2.01	1.02-3.97	2.07	1.26-3.39
BMI, kg/m ²	0.93	0.80-1.08	0.95	0.82-1.09
Glomerular filtration rate, mL/min	1.02	1.00-1.05	1.02	0.99-1.05
Low-dose vs standard dose DOAC	3.49	0.76-16.0	2.72	0.55-13.5
Antiplatelet treatment (yes vs no)	0.28	0.03-2.53	0.25	0.03-1.81

Both models were estimated using the Fine and Gray competitive risk regression model. The Akaike information criteria was 118.4 and 106.1 for the models using C-trough and C-peak, respectively.

The inclusion of enrollment center as a potential confounder was not significant ($P > 0.9$ for both models) and it is not reported because it did not materially change estimates.

CI, confidence interval; HR, hazard ratio.

standardized values in the lowest class compared with those in the higher classes, and hazard ratios and their 95% confidence intervals were calculated. The variable CHA₂DS₂VASc score (≥ 4 vs < 4) was arbitrarily categorized according to the median values as cutoff. Data were analyzed with the use of Prism software (version 9.3.1, GraphPad Software Incorporated, San Diego, CA) and SPSS software (version 11.0 SPSS Inc, IBM, Armonk, NY), and R (version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of patient population

The flowchart of the study population is shown in Figure 1. A total of 1718 patients, who started a DOAC treatment for nonvalvular AF, were included in the study. Ten patients were excluded for not meeting inclusion criteria ($n = 9$) or declining to participate ($n = 1$), and 51 were excluded because no blood sampling at steady state was available. A total of 1657 patients had blood sampling for DOAC level measurement, 15 to 30 days from the start of treatment (steady state). The clinical history for 1-year follow-up was

collected in 1345 patients and for a shorter period in 139 patients because the study was stopped (median follow-up, 302 days [minimum-maximum: 124-329]). A total of 173 patients were censored before 1 year of follow-up for the following reasons: they had changed the reference medical center ($n = 7$), the consent to the study was withdrawn ($n = 3$), the physician decided to change/stop the treatment ($n = 28$), they were lost to follow-up ($n = 26$), for thromboembolic or bleeding events ($n = 71$; including 9 deaths), and death due to other diseases ($n = 37$). The main demographic and clinical characteristics of the 1657 investigated patients are shown in Table 1.

Plasma samples for DOAC measurement were available for all patients at C-trough and in 1303 patients at C-peak. Results (mean $\pm$ standard deviation; and minimum/maximum) of DOAC levels, at C-trough and at C-peak, are shown in supplemental Table 1, in addition to the number of plasma samples analyzed for the different DOACs.

DOAC concentration levels and thrombotic events during follow-up

During a total follow-up of 1606 years, thromboembolic outcomes occurred in 21 patients (incidence of 1.31% pt/y): 6 strokes (1 fatal), 1 TIA, 1 VTE, 12 acute myocardial infarctions (5 fatal), and 1 superficial vein thrombosis were recorded. Details of patients who had thrombotic outcomes during follow-up are shown in Table 2. Altogether, 46 deaths were recorded (2.8%), 6 of whom were related to thrombotic complications (supplemental Table 2). DOAC plasma level was the most important independent predictor of the occurrence of the primary study outcome according to the best-fitting model (Table 3), even after adjustment for other possible confounders and enrollment centers. Patients with C-peak crude concentrations below the first quintile were particularly at risk of the primary study event (supplemental Table 3).

As shown in Figure 2, patients with thrombotic outcomes had C-trough DOAC values below the mean value for each drug in 17 (1.7% pt/y) cases, whereas 4 (0.69% pt/y) cases had values above the mean value. At C-peak, their values were below the mean in 13 (1.8% pt/y) cases and above in 4 (0.70% pt/y) cases. Using standardized C-trough values, patients were distributed into classes of increasing levels (Table 4). The highest incidence of thrombotic events (4.82% pt/y) occurred among 89 patients with

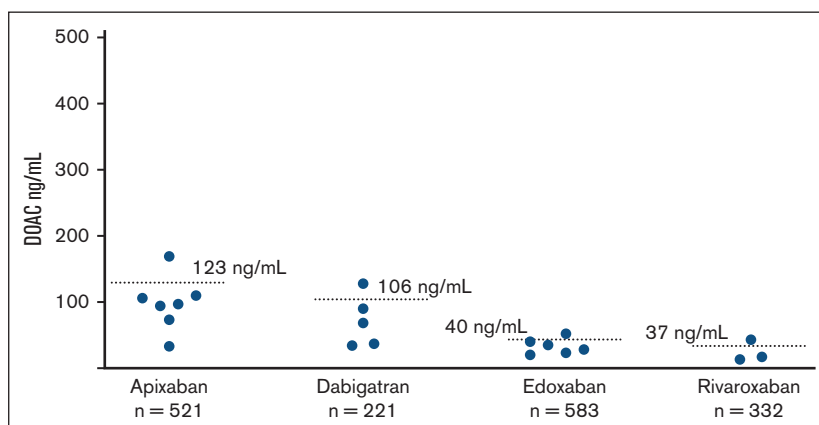
Figure 2. Measured C-trough DOAC levels assessed at steady-state in atrial fibrillation patients who experienced thrombotic outcomes within 1 year follow-up. Blue dots represent the values and dotted lines represent the mean values of each drug.

Table 4. Patient distribution in classes of standardized C-trough values around the mean value (0) for all anticoagulant drugs, with the number of patients and of thrombotic complications recorded in each class

Classes of standardized C-trough values	Equivalent DOAC C-trough plasma levels (ng/mL)										Thrombotic complication, n	Incidence, % pt/y (95% CI)
	Apixaban		Edoxaban		Rivaroxaban		Dabigatran					
	2.5 mg BID	5 mg BID	30 mg	60 mg	20 mg	110 mg BID	150 mg BID	Patients, n	Follow-up, y			
-1.00 or less	≤41	≤61	≤8	≤7	≤6	≤46	≤37	89	83	4	4.82 (1.3-12.3)	
From -0.99 to -0.50	44-77	63-94	9-23	8-24	8-22	51-78	38-68	442	430	7	1.63 (0.6-3.3)	
From -0.49 to 0	78-113	95-128	24-39	25-42	23-37	81-109	69-99	525	513	6	1.17 (0.4-2.5)	
From 0.01 to 0.50	114-146	129-160	40-54	43-58	38-52	115-138	101-128	279	277	3	1.08 (0.2-3.2)	
From 0.51 to 1.00	149-179	163-195	55-70	60-73	53-67	143-163	132-163	129	119	1	0.84 (0.02-4.7)	
>1.00	187-468	199-445	72-202	79-284	70-331	175-311	165-367	193	183	0	0	
	-	-	-	-	-	-	-	1657	1605	21	1.31 (0.8-2.0)	

The equivalent DOAC plasma levels at C-trough for each class are also reported.
BID, twice a day; CI, confidence interval.

standardized DOAC values in the lowest class (−1.00 or less) compared with the mean value; the incidence of events decreased sharply in the other classes. The incidence of events in the lowest class of standardized levels was significantly different than in the sum of all the other classes (4.82% pt/y vs 1.12% pt/y; $P = .0039$). The Kaplan-Meier curves of cumulative thrombotic outcomes occurring in patients in the lowest class of standardized DOAC levels, assessed at C-trough, compared with those in all the higher classes are shown in Figure 3.

Discussion

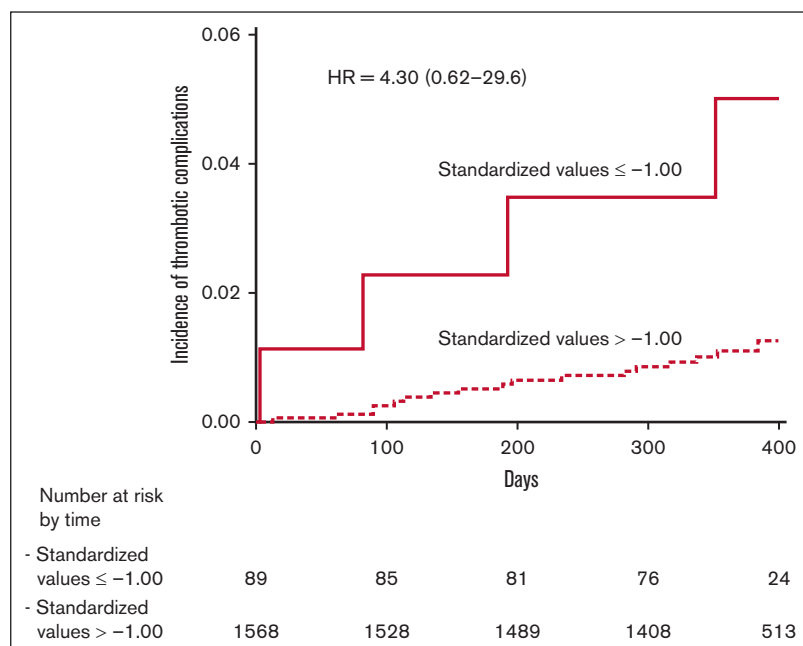
To our knowledge, the MAS study is the first observational, multi-center study that measured DOAC plasma levels in patients with AF at the beginning (steady state) of DOAC treatment who were prospectively followed-up to record thrombotic complications occurring within 1 year. DOAC levels were kept blind to patients and to attending physicians and merged with the individual patients only at the end of the study. The main findings of the study are that 17 of 21 patients who experienced thrombotic outcomes had low plasma activity of the drug at the beginning of treatment; furthermore, the highest incidence of thrombotic complications (4.82% pt/y) occurred in patients whose standardized levels were in the lowest and most distant class from the overall mean.

Efficacy of DOAC treatment for stroke prevention in AF has been widely documented⁵⁻⁹; however, a nonnegligible incidence of thrombotic events has been recorded in registration trials and clinical practice in patients receiving DOACs.⁹⁻¹¹ These results pose the question of how to improve clinical management of patients with AF treated with DOACs to further reduce the risk of complications. DOAC are administered to patients with AF at fixed dose, either standard or low dose in relation to patient characteristics (mainly age and renal function), without the need for laboratory monitoring. However, high interindividual variability has been confirmed in trials and observational studies,^{25,27-29,40-42} showing that some patients may have low anticoagulant levels and therefore are more exposed to an increased risk of thrombotic complications, especially if at high cardiovascular risk. In a previous study,³⁰ we observed a relationship between low DOAC levels and thrombotic events, particularly in patients with a high CHA₂DS₂VASc score. Very recently, a monocentric observational study³³ reported early stroke recurrence in 3% of patients with AF with a previous stroke, for whom low plasma levels of apixaban and dabigatran had been detected at steady state.

In this study, which involved 1657 patients with AF treated with the 4 DOACs currently on the market, we collected data on thrombotic cardiovascular events occurring for a 1-year follow-up after blood sampling taken at a steady state to measure the DOAC levels. We found that >80% of thrombotic complications occurred in patients with standardized values present in the classes below zero (which is the overall mean of DOAC levels). Interestingly, both C-peak and C-trough standardized DOAC plasma concentrations had similar predictive capability in our study; however, C-trough measurements are generally easier to obtain.

Although these results seem to indicate that measurement of DOAC levels at a steady state in patients with AF may help avoiding most thrombotic complications that may occur during treatment, our results clearly show that it would be unsuitable to measure the drug levels in all patients with AF who start a DOAC treatment to avoid (or reduce) the relatively few thrombotic

Figure 3. Cumulative event rates for thrombotic outcomes. The Kaplan-Meier cumulative event rates for the thrombotic outcomes in patients with DOAC levels in the standardized class of ≤ -1.00 or less (continuous line) and in patients with DOAC levels in the standardized classes of more than -1.00 (dotted line) at C-trough. HR, hazard ratio.



complications that were recorded during the 1-year follow-up (21 events, 1.31% pt/y). Nevertheless, it appears clinically relevant to try to improve the efficacy of DOAC treatment by further lowering the incidence of severe thrombotic complications, which would seem achievable by adjusting the daily dose or changing the drug in patients at high thrombotic risk who show low DOAC levels when measured at the beginning of treatment. To this aim, we propose that different combinations of patient characteristics might be helpful in identifying a criterion that would allow the minimum number of patients to be tested together with the maximum number of thrombotic events that potentially could be avoided.

Limitations

Our study has limitations. The enrollment was strongly affected by the COVID-19 pandemic, which dramatically blocked many activities at the participating centers. We admit that the study may present problems of generalizability because ~75% of patients were included in only 1 clinical center (Cremona). The 4 available DOACs are not similarly used in Italy and, consequently, the number of investigated patients was not equal for each drug. Although rivaroxaban tablets should be taken with food to increase absorption (product monograph, Bayer, revision April 2023), in this study blood sampling was performed before the administration of the subsequent DOAC dose, and we are not aware whether patient blood sampling was performed before or after food intake for individual patients; DOAC concentration was measured only once (ie, 15-30 days after the initiation of treatment). We therefore cannot exclude possible intraindividual changes in DOAC levels and problems in adherence to treatment during follow-up. However, a previous study reported that the intraindividual variability of DOAC levels was substantially lower than the interindividual variability.²⁸ In contrast, testing DOACs at different time points during follow-up would have been impractical. However, our study showed an association between very low DOAC levels assessed at the beginning of treatment and occurrence of thrombotic complications

during 1-year follow-up, and therefore our results were not influenced by possible DOAC level changes after the time point of measurement. It is well known that association with potent inducer agents of DOAC catabolism (such as antiepileptic drugs) may significantly reduce DOAC concentration.⁴³ The concomitant use of antiepileptic drugs in our study was reported in 28 of the investigated patients, 5 of whom had standardized C-trough values in the lowest class around the overall mean (-1.00 or less), and none of these patients had thrombotic complications during follow-up. All in all, we believe that an accurate analysis of the many factors that may interfere with DOAC concentration would be needed to determine which patients should be tested in future studies. For example, DOAC dosing is still uncertain in some groups of patients, such as those with an extreme body weight. Weight and BMI are important variables in drug distribution and plasma concentration levels, and in individuals with very low or high BMI, DOAC measurements may be considered.¹⁹ In our study population, the number of patients with BMI of <18.5 or >50 (criteria suggested by Steffel et al¹⁹ to define patients with extreme body weight) were small (39 and 1, respectively). The relatively low number of patients with extreme BMI may have contributed to the nonsignificant result of BMI as predictor of thrombotic outcomes in the competitive risk score analysis (Table 3).

The strengths of the study are its prospective and multicentric design; the centralization of laboratory tests, which avoided interlaboratory variations; and the blindness of all test results to patients and treating physicians, which avoided possible treatment adjustment during follow-up.

Future directions

Although we are aware that the results of this study are not sufficient to modify current clinical practice, we believe that our findings may pave the way to future studies aimed to definitively assess whether measuring DOAC plasma levels at steady state in selected patients may reduce the incidence of thrombotic complications during follow-up in the setting of patients with AF.

Conclusion

In conclusion, our data show a relationship between low DOAC levels measured at steady state and occurrence of stroke, TIA, VTE, and other thrombotic cardiovascular events in patients with AF. The results support the clinical utility of measuring DOAC concentration at the beginning of treatment in special settings of patients with AF. Laboratory measurement at steady state may allow to avoid a persistent treatment at insufficient DOAC concentration in patients who are at a very high thrombotic risk. Our results need to be confirmed and expanded with further clinical studies.

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Authorship

Contribution: S.T., G.P., and A. Tosetto conceptualized and designed the study; S.T., G.P., A. Tosetto, and C.L. analyzed and interpreted the data; S.T., G.P., and A. Tosetto drafted the article; S.T., G.P., A. Tosetto, C.L., and A. Tripodi critically revised the article for important intellectual content; O.P., A. Ciampa, D.P., R.M., M.T., P.C., R.C.S., A.M.I., E.D.C., P.P., E.M.F., A. Chistolini, M.d.P.E., and M.M. provided study materials or recruited patients; M.C., C.L., and E.A. provided administrative, technical, or logistic support; M.C., C.L., and C.D. collected and assembled data; and all authors approved the final version of the article.

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A complete list of the members of the MAS Study Group appears in "Appendix."

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